American Journal of Hematology 54:306–312 (1997)

Clonal Integration and Expression of Human T-Cell Lymphotropic Virus Type I in Carriers Detected by Polymerase Chain Reaction and Inverse PCR

Koichi Ohshima,[1]* Yasuo Mukai,[1] Hiroshi Shiraki,[2] Junji Suzumiya,[1] Koutaro Tashiro,[1] and Masahiro Kikuchi[1]

[1]Department of Pathology, School of Medicine, Fukuoka University, Fukuoka, Japan
[2]Fukuoka Red Cross Blood Center, Fukuoka, Japan

Adult T-cell leukemia (ATL) is a neoplasm of mature helper (CD4) T lymphocytes, and human T-cell lymphotropic virus type- I (HTLV-I) has been suggested to be the causative virus of ATL. HTLV-I integrates its proviruses into random sites in host chromosomal DNA. Clonal integration has been observed in patients with ATL, including smoldering, chronic, and acute states. However, random and/or polyclonal integration has only been reported in a few asymptomatic HTLV-I carriers. To clarify the clonality of HTLV-I-infected cells in carriers, we used an inverse polymerase chain reaction (IPCR), which is more sensitive than Southern blot analysis. We used the peripheral blood momonuclear cells (PBMC) from 16 asymptomatic carriers and the separated CD4-positive cells. No cases showed either a monoclonal or polyclonal integration of the HTLV-I provirus by Southern blot. But, using IPCR, 7 of 16 cases showed either mono- or oligoclonal integration. In addition, the populations of clonal provirus in the total PBMC were frequently different from those in the CD4-positive cells. Three cases showed expression of HTLV-I tax/rex mRNA in the total PBMC, but no such expression was found in CD4-positive cells. In this study, an unexpected frequency of clonal HTLV-I provirus DNA was observed in HTLV-I carriers. These findings indicate that the clonal but nonmalignant proliferation of HTLV-I-infected cells already occurs even in HTLV-I carriers, and therefore that some other step is necessary to induce malignant proliferation. Am. J. Hemato. 54:306–312, 1997.

Key words: HTLV-I carrier; HTLV-I provirus; PCR, inverse PCR

INTRODUCTION

Adult T-cell leukemia (ATL) is a human T-cell lymphotropic virus type I (HTLV-I)-associated T-cell malignancy. Its characteristic clinicopathologic features include a rapidly progressing clinical course, accompanied by the appearance of tumor cells with flower-like nuclei in the peripheral blood [1]. ATL can be diagnosed based on the clinicopathologic findings and the presence of monoclonal integrated proviral HTLV-I DNA in tumor cells with a helper/inducer (CD4) phenotype [2]. Clinically ATL is classified into three types: smoldering leukemia, chronic leukemia, and acute leukemia [3].

In HTLV-I carriers, the integration of the HTLV-I provirus is thought to be random. The random integration of the HTLV-I provirus was observed in some HTLV-I carriers by Southern blot analysis, thus indicating that the number of polyclonal HTLV-I-infected cells had increased. This is an intermediate[1] state, which is frequently associated with such immunodeficient states as strongyloidiasis [4]. The monoclonal expansion of HTLV-I-infected lymphocytes can be detected in the next step, i.e., smoldering ATL.

Inverse polymerase chain reaction (IPCR) is instrumental in the identification of unknown sequences flanking such known sequences as retroviral DNA. In addition, IPCR detected under 1% of ATL cells in HTLV-I-

Contract grant sponsor: Ministry of Education, Science, and Culture, Japan.

*Correspondence to: K. Ohshima, M.D., Department of Pathology, School of Medicine, Fukuoka University, Nanakuma 7-45-1, Jonan-ku, Fukuoka 814-01, Japan.

Received for publication 18 April 1996; Accepted 9 November 1996.

noninfected cells, thus indicating this assay to be more sensitive than Southern blot analysis [5].

A regulatory gene tax, which is located at the pX region of the HTLV-I genome, has been identified as the gene responsible for both viral replication and the abnormal growth and immortalization of HTLV-I-infected T cells. The tax protein transactivates the expression not only of the viral gene but also of the cellular genes, including the interleukin-2 receptor (IL-2R), the protooncogene (c-fos), and others. In fact, the expression of genes in the HTLV-I pX region has been shown to indicate immortalized normal T cells in an IL-2-dependent fashion. However, mRNA for tax genes was detected by using only very sensitive reverse transcription methods followed by the polymerase chain reaction (RT-PCR) [6].

To clarify the relationship between clonality and expression of tax in healthy HTLV-I carriers, we performed IPCR and RT-PCR.

MATERIALS AND METHODS

Cell Samples

Peripheral blood mononuclear cells (PBMCs) were obtained from 16 healthy HTLV-I carriers. Almost all cases were clinically followed for 4–5 years and were also healthy. The antibody against HTLV-I (adult T-cell leukemia antibody, ATLA) was measured in the sera using the gelatin-particle-agglutination (PA) and indirect immunofluorescence (IF) methods. The phenotype of the mononuclear cells in the peripheral blood cells was examined by monoclonal antibodies of Leu4 for CD3, of Leu3 for CD4, of Leu2 for CD8, and/or of Leu12 for CD19 (Becton-Dickinson, Sunnyvale, CA), using a FACScan analyzer (Becton-Deckinson).

A portion of the PBMCs were used to enrich CD4-positive cells (human CD4 cell recovery kit, Biotex Laboratories). Briefly, the kit is a rapid affinity chromatography tool. By a process of negative selection, virtually all B lymphocytes and CD8 cells were removed by column, leaving enriched CD4 cells in the column eluant. After enrichment, flow cytometric analyses was performed, and CD4 cells occupied >98% of all cells.

Southern Blot Analysis

A portion of the frozen material was used for DNA isolation and gene analysis. Details of the examination methods have been previously reported [7]. The proviral DNA of HTLV-I (full length: gag, pol, env, pX, and LTR) was examined by Southern blot analysis. DNA was digested with either restriction enzyme *Eco*RI or *Pst*I to analyze the monoclonal or polyclonal integration of HTLV-I, while the T-cell receptor (TCR) genes Cβ and Jγ were examined for the monoclonal population.

TABLE I. Primers and Oligoprobes

Primer/probe		5′→3′sequence
pX1	(primer)	CGATCACGATGCGTTTCCCC
pX2	(primer)	ATTATCCTTGGAGGAAGAGT
pX	(probe)	GCTTTCCTCTTCTAAGGATAGC
RPX3	(primer)	ATCCCGTGGAGACTCCTCAA
RPX4	(primer)	AACACGTAGACTGGGTATCC
RPXPRI	(probe)	AACACCATGGCCCACTTCCC
U5-1	(primer)	AAGCCGGCAGTCAGTCGTGA
U5-2	(primer)	AAGTACCGGCAACTCTGGTG
U5-3	(primer)	GAAAGGGAAAGGGGTGGAAC
U5-4	(primer)	CCAGCGACAGCCCATTCTAT
U5-5	(probe)	CTCCAGGAGAGAAATTTAGTACAC

Polymerase Chain Reaction

Isolated DNA was used for polymerase chain reaction (PCR) with specific primers synthesized based on published DNA sequences. For HTLV-I, primers pX-1 and pX-2 were synthesized, corresponding to the pX region of HTLV-I [8]. Amplification was done with the GeneAmp DNA amplification reagent kit and the DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, CT). After 30 cycles of PCR amplification, we analyzed one tenth of the reaction mixture (10 μl) by Southern blot methods, using an oligoprobe.

Reverse Transcription PCR

Total RNA was prepared from frozen materials and PBMCs by the guanidium thiocyanate-phenol-chloroform method, using a Total RNA Separator kit (Clontech Laboratories, Palo Alto, CA). We used 250 ng of the RNA preparation for RT-PCR (Thermostable rTth Reverse Transcriptase RNA PCR kit, Perkin-Elmer Cetus). To detect HTLV-I tax1/rex1 mRNA, we synthesized primers RPX3 and RPX4, based on the findings of a previous report [6]. In addition, the oligoprobe was also synthesized.

Inverse PCR

Details of the examination methods have been previously reported [5]. Briefly, DNA was digested with *Sau*3AI or *Alu*I and then ligated to cause self-ligation. The ligated DNA was then digested with *Sac*II to eliminate the circular DNA originating from the 5′ proviral DNA. This DNA was then used as a template. For the first PCR, primers U5-1 and U5-2 were used. In addition, some of the PCR products were used for the next PCR with the nested primers U5-3 and U5-4. After PCR amplification, we analyzed the PCR products by Southern blot methods, using an oligoprobe (U5-5). The oligoprimers and probes are summarized in Table I.

TABLE II. Clinical and Marker Studies*

Patient no.	Age	Sex	WBC (/mm³)	Aty (%)	RBC (x 10⁴/mm³)	Plt	GOT (IU)	HTLV-I (PA/IF)	HIV (PA)	CD3 (%)	CD4 (%)	CD8 (%)	(CD4/8)	CD19 (%)
1	61	M	5,100	/	522	28.7	12	+6/+	−	31	24	15	(1.6)	7
2	41	F	5,600	/	444	16.1	12	+6/+	−	28	16	13	(1.2)	13
3	35	M	8,400	/	504	24.4	11	+6/+	−	/	/	/	/	/
4	45	F	4,800	0.5	473	22.1	19	+6/+	−	/	/	/	/	/
5	43	F	/	0	/	/	27	+6/+	−	26	22	21	(1.0)	23
6	54	F	5,500	0	442	35.1	6	+6/+	−	43	28	17	(1.6)	24
7	59	M	6,900	1	528	18.3	10	+6/+	−	34	36	40	(0.9)	15
8	39	F	7,300	0	432	22.5	7	+6/+	−	51	34	23	(1.5)	28
9	42	M	6,300	1.5	403	16.6	5	+6/+	−	77	30	64	(0.5)	22
10	47	F	/	0	/	/	16	+6/+	−	62	39	40	(1.0)	16
11	35	M	/	0	/	/	31	+6/+	−	57	41	28	(1.5)	33
12	59	M	6,700	1	465	23.5	7	+6/+	−	68	51	49	(1.0)	27
13	49	M	7,400	0	457	30.3	7	+6/+	−	25	20	10	(2.0)	30
14	52	M	/	0	/	/	9	+6/+	−	50	33	29	(1.1)	19
15	17	M	/	0	/	/	6	+6/+	−	60	31	25	(1.2)	39
16	54	F	/	0	/	/	14	+6/+	−	74	38	30	(1.3)	15

*slash = not done; Aty, atypical lymphocyte; GOT, glutamic oxaloacetic transaminase. Normal range for healthy controls: WBC, 3,500–9,700; RBC, 376–577; GOT, 10–40; CD3, 40–82; CD4, 22–55; CD8, 11–48; CD4/CD8, 0.5–3.0; CD19, 5–35.

RESULTS

Laboratory Data

Sixteen cases (9 males, 7 females), age 17–61 years, who showed no clinical data related to the manifestations of ATL were studied. All had antibodies to HTLV-I but were healthy. Four cases had atypical lymphocytes (0.5–1.5%) on routine hematological examination. The atypical lymphocytes showed mild nuclear atypia, but were different from typical ATL flower-like cells. A phenotypic analysis of the peripheral blood lymphocytes was performed, and the absolute numbers of CD3, CD4, CD8, and CD19, and the CD4/CD8 ratio, were almost in normal range (Table II).

Detection of HTLV-I Provirus

No cases showed either a monoclonal or polyclonal integration of the HTLV-I provirus by Southern blot analysis (Fig. 1). In addition, no samples with CD4-positive cells demonstrated either monoclonal or polyclonal integration. All samples with both PBMCs and CD4-positive cells showed an amplification of the HTLV-I provirus by PCR (Table III, Fig. 2). Using Southern blot analysis, we could detect about 5% of monoclonal cells with HTLV-I integration (data not shown).

Clonality of HTLV-I-Infected Cells

By IPCR, 7 of 16 cases with total PBMCs showed either mono- or oligoclonal integration (Fig. 2). In the 7 cases with clonal bands, one monoclonal band was found in 4 cases, two clonal bands were seen in 1 case, and three clonal bands were observed in 2 cases. In addition, 3 of 14 cases with CD4-positive cells showed either mono- or oligoclonal integration. All 3 cases showed two clonal bands. In addition, 3 cases showed clonal bands in both PBMCs and CD4-positive cells, and 4 cases showed bands only in PBMCs, but none in CD4-positive cells. In summary, by IPCR, 9 cases showed no bands, 4 cases showed clonal bands only in total PBMCs, and 3 showed clonal bands both in total PBMCs and CD4-positive cells.

In addition, the lengths of clonal bands in PBMCs were frequently different from those in CD4-positive cells. The different lengths of clonal bands signified the presence of different clonal cell populations with HTLV-I integration. Different clones between the CD4-positive cells and total PBMCs signified that HTLV-I integrated non-CD4-positive cells. Using IPCR, we could detect roughly 0.5–1% of monoclonal cells with HTLV-I integration (data not shown).

Tax/rex Expression

Three cases showed expression of HTLV-I rax/rex mRNA in PBMC, but no expression was found in CD4-positive cells. The 3 cases with tax/rex expression showed clonal bands in IPCR (Fig. 2).

TCR Gene Analysis

The oligoclonal rearranged bands of TCR Cβ were found in 4 of 16 cases with PBMCs, and in 8 of 13 cases with CD4-positive cells. In addition, the rearranged bands of CD4-positive cells showed a more increased density than those in PBMCs (Fig. 1). The oligoclonal rearranged bands of TCR Jγ were found in 13 of the 16 cases with PBMCs, and in all cases with CD4-positive cells. In addition, the rearranged bands of CD4-positive

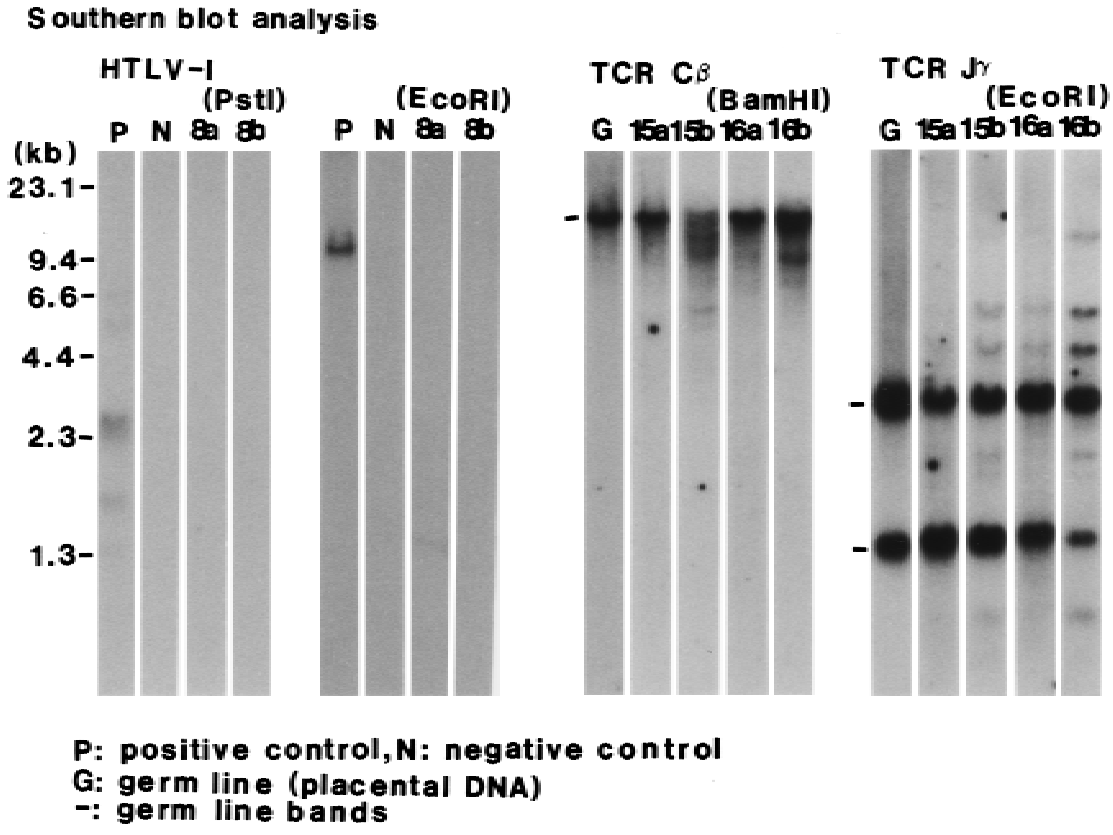

Fig. 1. Southern blot analysis. Lane number corresponds to case number (a: total DNA of PBMCs; b: DNA of CD4-positive cells). In HTLV-I analysis, 10 μg of DNA were digested with *Eco*RI or *Pst*I. There were no proviral HTLV-I DNA bands in any of the cases. The positive control was typical ATL. Polyclonal faint, rearranged bands were found in the T-cell receptor Cβ and Jγ chain genes. Bands of polyclonal rearrangements of CD4-positive cells (b) increased in number and density, rather than those of PBMCs (a).

cells also showed an increased density and greater number than those in the PBMCs (Fig. 1). In summary, it was shown that oligoclonal populations of T cells were found in all HTLV-I carriers, especially in those with CD4-positive cells.

DISCUSSION

ATL is a monoclonal neoplasm of mature helper (CD4) T lymphocytes, and shows a monoclonal integration of the HTLV-I provirus [2]. However, HTLV-I infects not only CD4+ T cells but also CD8+ T cells, monocytes, and B cells in both ATL and healthy carriers [9]. In addition, the findings of this study support the fact that clonal HTLV-I-infected cells are found not only in CD4+ cells, but in other cells as well.

The clinical stage in the peripheral blood gradually progresses from carrier to smoldering, chronic, and finally acute-type leukemia. The model of HTLV-I proviral DNA integration also changes from undetectable to polyclonal, and then to monoclonal malignant transformation [10]. In addition, leukemic cells were always monoclonal in respect to proviral integration, which indicated that they originated from a single cell infected with HTLV-I [10].

In HTLV-I carriers, integration of the HTLV-I provirus is thought to be random. The random integration of the HTLV-I provirus was observed in some HTLV-I carriers by Southern blot analysis, thus indicating that the number of polyclonal HTLV-I-infected cells had increased. This is considered an intermediate state, frequently associated with such immunodeficient states as strongyloidiasis [4]. The monoclonal expansion of HTLV-I-infected lymphocytes can be detected in the next step, i.e., smoldering ATL [4]. Our cases are all believed to be in the carrier state, and not in the intermediate state.

Southern blot analyses with HTLV-I probes were used to detect the monoclonality of the integrated HTLV-I provirus. However, IPCR is more sensitive than Southern blot analysis. It can detect fewer than 1% monoclonal cells, whereas a Southern blot analysis needs about 5% monoclonal cells [5]. Previously, IPCR could detect the monoclonal proliferation of HTLV-I-infected cells in an intermediate state, as well as in smoldering, chronic, and acute-type leukemia. However, no clonal bands were de-

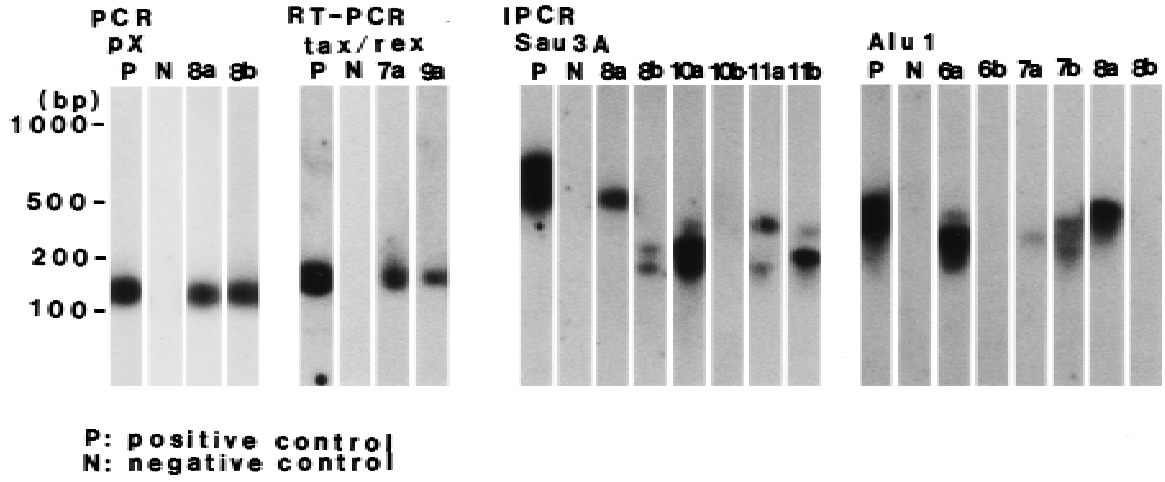

Fig. 2. PCR analysis. Lane number corresponds to case number (a: total DNA; b: DNA of CD4-positive cells). Samples with both PBMCs and CD4-positive cells showed amplification of the HTLV-I provirus by PCR. By RT-PCR, the expression of HTLV-I tax/rex mRNA was found in the DNA of PBMCs, but not in the CD4-positive cells. Using IPCR, the samples of total PBMCs showed either mono- or oligoclonal integration, and those of CD4-positive cells also showed either mono- or oligoclonal integration. But the lengths of clonal bands in PBMCs frequently differed from those in CD4-positive cells.

tected in any carriers [5]. In our study, meanwhile, 7 of 16 carriers showed oligoclonal integration. This indicated that clonal but nonmalignant proliferation of HTLV-I-infected cells had already occurred, even in HTLV-I carriers.

In Japan, the annual incidence of ATL among carriers has previously been estimated to be around 0.06% [11,12]. In a large-scale molecular detection of monoclonal integration in a population (481 cases) of healthy carriers, Southern blot analysis showed monoclonal integration in 6 cases (1.2%), and all 6 cases were older than 40 years. These results indicate that the molecularly detectable pre-ATL also develops after a long latency [13]. However, in our IPCR study, clonal integration was not confined to people older than 40. The cause is probably due to the sensitivity of IPCR, which can detect a small amount of clonal integration.

The HTLV-I genome has an extra sequence, the so-called pX region, which encodes the trans-acting factors, Tax and Rex [8]. Tax is also a trans-activator for the transcription of such cellular genes as interleukin-2 (IL-2), and interleukin-2 receptor (IL-2R) α [14,15]. The primary event after HTLV-I infection is believed to be the IL-2/IL-2 receptor autocrine loop, which thereafter leads to an immortalized state [16]. Tax/rex expression was detected in the PBMCs of ATLL patients and asymptomatic HTLV-I carriers, although the amounts of mRNA detected corresponded to 10^4–10^6 times less than in the HTLV-I-infected MT-2 cell line [6]. The level of tax1/rex1 mRNA expression was not correlated with the number of atypical lymphocytes. These findings are consistent with the idea that the expression of one or more HTLV-I genes may be involved in initiating the transformation, but no consistent expression is needed for the leukemogenesis of immortalized cells [6]. In this study, the cases with tax/rex expression showed clonal bands in IPCR. This finding might thus be related to the first step of clonal expansion of HTLV-I-infected cells.

The tumor-infiltrating lymphocytes (TILs) in lung cancer frequently exhibit monoclonal or oligoclonal TCR Cβ rearrangements [17]. In this study, using Southern blot analysis, oligoclonal rearrangements were obtained; however, no clonal or polyclonal HTLV-I DNA integration was observed. These results indicate that the clonally expanded T-cell populations without HTLV-I infection frequently exist in the PBMCs of HTLV-I carriers.

The cytotoxic T lymphocytes (CTL) derived from a patient with HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) expressed CD8 antigen or CD4 antigen [18,19]. They showed a cytotoxic effect predominantly against the target cells expressing HTLV-I $p40^{tax}$. In contrast, the CTL, induced from an asymptomatic HTLV-I carrier, possessed CD4 antigen [18]. In this study, the CD4/CD8 ratio was also observed not to increase. However, oligoclonal T-cell expansion was detected, especially in CD4-positive cells. These findings may support the hypothesis that immunity for HTLV-I is related mainly to CD4-positive cells in a carrier state [18].

There seem to be a higher number of T cells with a monoclonally integrated provirus. However, we could not determine whether HTLV-I induces enhanced proliferation at an early time point during infection or do antigen-specific T-cell proliferation, which happens to carry an integrated provirus.

TABLE III. DNA and RNA Analysis*

	Southern blot, HTLV-I		TCR		PCR	Inverse-PCR		RT-PCT
	EcoRI	Pst I	Cβ	Jγ	pX	Sau3A	AluI	tax/rex
1a	−	−	G	5	+	−	−	−
2a	−	−	P-R	8	+	−	−	−
3a	−	−	p-r	6	+	−	−	−
3b	−	−			+	−	−	−
4a	−	−	p-r	5	+	−	−	−
4b	−	−	P-R	6	+	−	−	−
5a	−	−	p-r	0	+	−	−	−
5b	−	−	P-R	4	+	−	−	−
6a	−	−	G	0	+	−	2	−
6b	−	−	p-r	4	+	−	−	−
7a	−	−	p-r	2	+	−	1	+
7b	−	−	p-r	7	+	−	2	−
8a	−	−	G	3	+	1	1	−
8b	−	−	G	6	+	2	−	−
9a	−	−	G	6	+	1	−	+
9b	−	−	G	6	+	−	−	−
10a	−	−	G	4	+	3	−	−
10b	−	−	p-r	6	+	−	−	−
11a	−	−	G	3	+	1	−	−
11b	−	−	G	6	+	−	−	−
12a	−	−	G	5	+	3	1	+
12b	−	−	G	6	+	2	−	−
13a	−	−	G	0	+	−	−	−
13b	−	−	G	5	+	−	−	−
14a	−	−	G	2	+	−	−	−
14b	−	−	G	6	+	−	−	−
15a	−	−	G	2	+	−	−	−
15b	−	−	P-R	6	+	−	−	−
16a	−	−	G	4	+	−	−	−
16b	−	−	p-r	6	+	−	−	−

*a, total DNA; b, DNA of CD4-positive cells; P-R, large population of polyclonal rearrangements; p-r, small population of polyclonal rearrangements; G, germ line. Number represents number of bands of rearrangements or intergrated proviruses.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid for the Encouragement of Young Scientists from the Ministry of Education, Science, and Culture, Japan.

REFERENCES

1. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H: Adult T-cell leukemia. Clinical and hematologic features of 16 cases. Blood 50: 481–492, 1977.
2. Yoshida M, Seiki M, Yamaguchi K, Takatsuki K: Monoclonal integration of human T-cell leukemia provirus in all primary tumors of adult T-cell leukemia suggests causative role of human T-cell leukemia virus in the disease. Proc Natl Acad Sci USA 81:2534–2537, 1984.
3. Kawano F, Yamaguchi K, Nishimura H, Tsuda H, Takatsuki K: Variation in the clinical courses of adult T-cell leukemia. Cancer 55:851–856, 1985.
4. Yamaguchi K, Kiyokawa T, Nakada K, Yul LS, Asou N, Ishii T, Sanada I, Seiki M, Yoshida M, Natutes E, Catovsky D, Takatsuki K: Polyclonal integration of HTLV-I proviral DNA in lymphocytes from HTLV-I seropositive individuals: An intermediate state between the healthy carrier state and smouldering ATL. Br J Haematol 68:169–174, 1988.
5. Takemoto S, Matsuoka M, Yamaguchi K, Takatsuki K: A novel diagnostic method of adult T-cell leukemia: Monoclonal integration of human T-cell lymphotropic virus type I provirus DNA detected by inverse polymerase chain reaction. Blood 84:3080–3085, 1994.
6. Kinoshita T, Shimoyama M, Tobinai K, Ito M, Ito S, Ikeda S, Tajima K, Shimotohno K: Detection of mRNA for the tax1/rex1 gene of human T-cell leukemia virus type I in fresh peripheral blood mononuclear cells of adult T-cell leukemia patients and viral carriers by using the polymerase chain reaction. Proc Natl Acad Sci USA 86: 5620–5624, 1989.
7. Ohshima K, Kikuchi M, Masuda Y, Sumiyoshi Y, Eguchi F, Mohtai H, Takeshita M, Kimura N: Human T-cell leukemia virus type I associated lymphadenitis. Cancer 69:239–248, 1992.
8. Seiki M, Hattori S, Hirayama Y, Yoshida M: Human adult T-cell leukemia virus: Complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. Proc Natl Acad Sci USA 80:3618–3622, 1983.
9. Koyanagi Y, Itoyama Y, Nakamura N, Takamatsu K, Kira J, Iwamasa T, Goto I, Yamamoto N: In vivo infection of human T-cell leukemia virus type I in non-T cells. Virology 196:25–33, 1993.
10. Takatsuki K, Yamaguchi K, Watanabe T: Adult T-cell leukemia and HTLV-I related diseases. Gann Mono Cancer Res 39:1–15, 1992.
11. Tajima K, Kamura S, Ito M, Nagatomo M, Kinoshita K, Iketa S: Epidemiological features of HTLV-I carriers and incidence of ATL in an ATL-endemic island: A report of the community-based cooperative study in Tsushima, Japan. Int J Cancer 40:741–746, 1987.

12. Kondo T, Kono H, Miyamoto N, Yoshida R, Toki H, Matsumoto I, Hara M, Inoue H, Inatsuki A, Funatsu T, Yamano N, Bando F, Iwao E, Miyoshi I, Hinuma Y, Hanaoka M: Age- and sex-specific cumulative rate and risk of ATLL for HTLV-I carriers. Int J Cancer 43:1061–1064, 1989.
13. Chen YX, Ikeda S, Mori H, Hata T, Tsukasaki K, Momita S, Yamada Y, Kamihira S, Mine M, Tomonaga M: Molecular detection of pre-ATL state among healthy HTLV-I carriers in an endemic area of Japan. Int J Cancer 60:798–801, 1995.
14. Siekevitz M, Feinberg MB, Holbrook N: Activation of interleukin 2 and interleukin 2 receptor (Tac) promotor expression by the trans-activator (tat) gene product of human T-cell leukemia virus type I. Proc Natl Acad Sci USA 84:5389–5393, 1987.
15. Fujii M, Sassone-Corsi P, Verma IM: c-fos promotor trans-activation by the tax1 protein of human T-cell leukemia virus type I. Proc Natl Acad Sci USA 85:8526–8530, 1988.
16. Yoshida M, Fujisawa J: Positive and negative regulation of HTLV-I gene expression and their roles in leukemogenesis in ATL. Advances in adult T-cell leukemia and HTLV-I research. Gann Monogr 39:217–235, 1992.
17. Yoshino I, Yano T, Yoshikai Y, Murata M, Sugimachi K, Kimura G, Nomoto K: Oligoclonal T lymphocytes infiltrating human lung cancer tissues. Int J Cancer 47:654–658, 1991.
18. Kannagi M, Harada S, Maruyama I, Inoko H, Igarashi H, Kuwashima G, Sato S, Morita M, Kidokoro M, Sugimoto M, Funahashi S, Osame M, Shida H: Predominant recognition of human T cell leukemia virus type I (HTLV-I) pX gene products by human CD8+ cytotoxic T cells directed against HTLV-I-infected cells. Int Immunol 8:761–767, 1991.
19. Jacobson S, Seuben JS, Streilein RD, Palker T: Induction of CD4+, human T lymphotrophic virus type-I-specific cytotoxic T lymphocytes from patients with HAM/TSP. J Immunol 146:1155–1162, 1991.